Live-Dead Bacterial Staining Kit

Introduction

The Live-Dead Bacterial Staining Kit provides an assay for detecting live bacteria and dead bacteria at the same time. The kit contains two dyes, NucGreen and EthD-III. NucGreen is a green nucleic acid dye that can stain both live and dead bacteria; EthD-III is a red nucleic acid dye that only stains bacteria with damaged cell membranes, such as dead bacteria. The viability of bacteria can be identified through the kit, with healthy bacteria appearing green and dead bacteria having a superposition of green and red.

Components and Storage

Components	K2239-20 T	K2239-100 T
NucGreen	20 μL	100 μL
EthD-III	40 μL	200 μL
Store at -20°C away from light, avoiding repeated freeze and thaw cycles, stable for 6 months.		

Protocol

1. **Bacterial sample preparation:**

   1) Grow the bacteria in a suitable liquid medium to the late logarithmic growth.

   2) Take 1 mL of bacterial solution and centrifuge at 5000-10000 g for 10-15 min.

   3) Remove the supernatant and resuspend the bacteria by adding 1 mL of 0.85% NaCl solution to the tube. Centrifuge at 5000-10000 g for 10-15 min and remove the supernatant. Add 1 mL of 0.85% NaCl solution to resuspend the bacteria and repeat the centrifugation once.

   4) Resuspend the bacteria with 0.85% NaCl solution and adjust the cell density to 10^8 bacteria/mL (OD_{670} ≈0.03）。

2. **Bacterial staining:**

   1) Take 1 μL of NucGreen, 2 μL of EthD-III, and 3 μL of 0.85% NaCl solution, and mix thoroughly to make the bacterial staining working solution.

   2) Add 5 μL of staining working solution into 100 μL of the bacterial solution, mix thoroughly, and incubate for 15 min at room temperature in the dark.

3. **Detection:** After staining, 10 μL of bacterial solution can be added to the clean slide, and apply a glass coverslip. The detection conditions for the two dyes are:

   NucGreen: Ex/Em: 503/530 nm (after binding to DNA)

   EthD-III: Ex/Em: 530/620 nm (after binding to DNA)

***Note:** When observing through fluorescence microscopy, the fluorescence signals of these two dyes can be observed using FITC and Cy3 (or Texas Red) filters, respectively.

Note

1. If the plates are used for detection, a small amount of bacterial solution can be left for imaging after standing for 10 min, which can effectively reduce the background.
2. It is recommended to keep the red and green fluorescence brightness consistent when merging.
3. Growth media should be removed prior to bacterial staining. Nucleic acids or other media components may bind to the dyes in some way, affecting staining results. Phosphate buffer (PBS) is not recommended in experiments to avoid reducing staining efficiency.
4. It is best to adjust the concentration of dyes appropriately according to different strains, and generally try to use the lowest dye concentration that can provide sufficient signal.
5. For your safety and health, please wear lab coats and gloves during the experiment.
6. For research use only. Not to be used in clinical diagnostic or clinical trials.

APExBIO Technology
www.apexbt.com
7505 Fannin street, Suite 410, Houston, TX 77054.
Tel: +1-832-696-8203 | Fax: +1-832-641-3177 | Email: info@apexbt.com